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Department of Agriculture Federated Malay States

Bulletin No. 16.

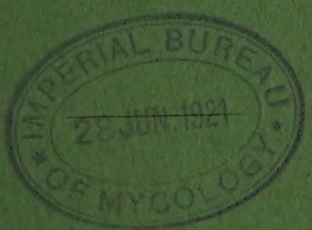
THE SPOTTING OF PLANTATION PARA RUBBER.

(A PRELIMINARY ACCOUNT OF INVESTIGATIONS ON THE CAUSE OF THE SPOTTING.)

BY

KEITH BANCROFT, M.A. (Cantab), F.L.S.

Mycologist, F.M.S.



Kuala Lumpur, Federated Malay States
January, 1913.

Department of Agriculture, F.M.S.

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PREFACE.

The spotting of the prepared plantation rubber which has been much in evidence in the Federated Malay States during the past three years has been attributed previously to various causes among which are the occurrence of chemical changes in the rubber and the growth of bacteria. The present Bulletin, No. 16, deals with the work which has been carried out on the spotting and demonstrates that the commoner causes are growths of minute fungi. A bacillus is also shown to be capable of producing spotting. The relative value of different methods of preventing the production of spots is also dealt with.

L. LEWTON-BRAIN,

Director.

THE SPOTTING OF PLANTATION PARA RUBBER.

By Keith Bancroft, m.a. (Cantab), f.l.s.
Mycologist, F. M. S.

INTRODUCTION.

The occurrence of spots or local discolourations in prepared plantation rubber has been recorded from several of the rubber producing countries of the East. The first publication on their occurrence appears to have been from Ceylon in 1910, where Petch described certain clear red spots on crepe and biscuits and reported the occurrence of black spots on wet biscuits.

In Sarawak Brooks recorded crimson spots on crepe in 1910; he reported as follows:—"during 1910 my attention was called to the occurrence of crimson spots on Para crepe which developed during drying.....the infection lasted for a month to six weeks and disappeared almost as suddenly as it had appeared, without the certain source of contamination being discovered."

In Burma blood-red spots have been recorded on prepared rubber.

In the Federated Malay States the history of the spotting on plantation rubber appears to date back to 1907, when an out-break of spots is said to have occurred; several persons have recollections of spotted rubber during that year, but subsequently there was evidently a considerable diminution in the quantity of the spotted rubber, and it has been difficult to obtain further record of the spotting until towards the end of 1910. From the result of careful enquiry, however, it would appear that a spotting of the lower grades of plantation rubber was observed on several occasions on estates between 1907 and 1910. During the early part of 1911 the spotted rubber increased considerably in quantity and was particularly abundant in February and March of that year. Towards the close of the year it appeared to diminish considerably and to disappear altogether from some estates. During the early part of 1912 spotted rubber was not so much in evidence as it was during 1911; but towards the end of the year there were indications of an increase in the quantity of spotted rubber.

During 1911 large quantities of spotted rubber were exported by plantations in the Federated Malay States. Estates producing crepe rubber which was altogether free from spotting appear to have been in the minority.

The cause of the spotting was for some time quite obscure. An early suggestion was that it was due to the alteration of chemical bodies in the rubber, or in the latex before coagulation. It appears to be satisfactorily established now that the presence of a blackening in latex and in the rubber which coagulates on the tree is due to the oxidation of certain chemical bodies.* One would however expect discolourations which are produced by chemical changes of such a nature to be more generally distributed through the rubber after its preparation and not to appear here and there as isolated, local discolourations. Further, on biscuits and on sheet rubber, a distinctly radial spread of the discolouration from a central point is sometimes visible, such as one would expect to be due to the growth of an organism.

Other suggestions were later made as to their cause. Carruthers, according to Petch, attributed them to a mould, a species of *Syncephalis*, growing on the rubber; while Ridley suggested that the red spotting was due to an alga, *Protococcus nivalis*, which colours the snow red in Europe.

Petch reported that an examination of the red spots in Ceylon did not show the presence of any organism to which they could be attributed. He considered that they were not due either to a fungus or to an alga, and that although they were such as would be expected to be caused by bacteria the presence of bacteria had not been demonstrated. On the other hand he stated that the black spots which occurred on wet biscuits contained numerous bacteria along with a black pigment, and he regarded the colouring matter as being produced by the bacteria.

In January 1911 Brooks reported that he had made inoculations from crimson spots on crepe and had obtained on sterilised bread and agar-agar an organism in crimson culture which he considered to be *Bacillus prodigiosus*. This bacillus is responsible for the occurrence of red spots on bread, meat and other articles of food.

Samples of a red spotting which occurred on sheet at Kuala Lumpur were sent home in 1911 by Sidney Morgan, Resident Chemist to the Rubber Growers' Association, and were reported to be due to *Bacillus prodigiosus*.

* The Preparation of Plantation Para Rubber; by H. J. Eaton, Bull. No. 17, Dept. of Agr. F.M.S.

In October 1911 a note on certain blue and red spots by the author was published in the *Agricultural Bulletin of the Straits Settlements and Federated Malay States* to the effect that what appeared to be fungus hyphae had been demonstrated in some of the spots and were probably the cause of the discolourations.

Following on the above work with respect to the presence of microorganisms, attempts were made to isolate the organisms from the spots with a view to ascertaining whether any of the discolourations could be definitely attributed to them; and the work which has been carried out has led to certain conclusions being drawn as the relative value of different measures for preventing or diminishing the occurrence of spots in prepared rubber.

THE APPEARANCE OF THE SPOTS.

Spots of several different hues have been recorded on sheet and crepe rubber in the Federated Malay States during the past three years. Generally speaking, the shades are bright red, pink, reddish yellow, dark blue, bluish green and bright yellow; while black and also clear spots have been recorded. The colours are usually more clearly visible if the spotted rubber be held up to the light.

If the rubber is cut across after it has dried the discolourations in many cases are found to penetrate throughout its entire thickness; in other cases they may occupy only a portion of the thickness of the rubber. Sometimes the discolouration may be present only on the upper and lower surfaces of the rubber, the middle portion being colourless.

The spots have been found to vary in size from small specks of about 1 or 2 mm. in diameter to large blotches as much as 15 cm. in width.

In the red and blue spots the discolouration has frequently been found to proceed from a central darker portion which appears as a nucleus.

In the red discolourations only has a diffuse colouring been found to occur and this has been observed on sheet where the discolouration takes the form of a flush measuring as much as 15 cm. in width.

Sometimes dark brown or black discolourations are found on crepe extending for some distance along the edges of the crepe.

In some cases where the spots are particularly abundant the rubber appears mottled red and yellow throughout the greater part of its surface area.

Whereas a large number of estates in this country have produced spotted rubber, the quantity of spotting has been found to vary greatly not only on different estates, but in the same factory at the same time. Sometimes only one or two spots are visible on a length of three feet of sheet or crepe. On some samples the spots have been so numerous as to occupy the greater part of the area of the rubber. Brooks recorded as many as 288 spots on a square foot of crepe. On one occasion a sample of crepe which was received by this Department was mottled red and reddish yellow throughout and was said to have been taken from a batch of 1,400 lbs. which was all equally badly affected.

While in several factories rubber has been found to be spotted continuously for some months, in some cases the spotting has affected only 2 or 3 weeks' out-put. Again, the spotted rubber has in a few cases been found to be limited to the product of the latex which was derived from only one or two fields of the Estate. There is accordingly a considerable variation in the production of the spotting.

Certain forms of prepared rubber have been found to be more susceptible to spotting than others; while some forms have never shown spotting. The following, which is obtained from notes on observations, will indicate what has been found to obtain in the different forms of rubber which are in common preparation in the country:—*Sheet, smoked,* sheet has not been found to develop spots when it is smoked. In some cases spotted sheets have been smoked after the spots appeared; in such cases the spots are not obliterated but are rendered less evident. *Sheet unsmoked,* red, blue and black spots have been recorded; the first has been fairly commonly observed, the other two much less frequently. *Crepe smoked,* as in smoked sheet. *Crepe thin,* red, yellow, blue, green and black spots have been recorded. *Crepe thick,* with the exception of "blanket" crepe which is usually made by rolling together separate sheets of thin crepe, thicker crepe appears to be more susceptible to spotting than thin crepe. *Crepe steamed,* very susceptible to spotting, more particularly the deep red and blue spots. *Crepe dried artifically,* has not been observed to develop spots. *Crepe bark,* red and reddish yellow spots have been commonly observed.

The spots commence to appear when the rubber is drying. In crepe they may become visible at the end of a week or may take a longer period to develop. On sheet the spots have been found usually to take longer to appear than on crepe. There are records however, in which both sheet

and crepe have developed red discolourations within 3 or 4 days after rolling. The discolourations become more apparent and may increase both in size and number until the rubber is dry, when any further increase does not usually occur.

The question arises as to whether spots may appear or disappear when the rubber is in transit. I have on record that on one occasion a batch of about 9 lbs. of a case of thin crepe was found to be discoloured bright red on arriving in London and was supposed to have been packed and shipped dry and unspotted. Several observers have considered that the spots may appear when the rubber is in transit. It is, however, questionable as to whether or not small quantities of spotted rubber may not escape notice in packing.

With regard to the disappearance of the spots while the rubber is in transit the following results on keeping spotted rubber in the laboratory may be given:—"Samples of spotted rubber have been kept in the laboratory for various periods of time extending up to eight months for the purpose of ascertaining whether the discolourations were retained for a long period. The samples consisted of sheet and crepe with red, blue, yellow and black spots, several samples of each being preserved. It has been found that the red colour on sheet and on crepe may sometimes fade slightly after several weeks. Two samples which were preserved showed signs of fading at the end of about four or five weeks. The red colouring has become less obvious, but it can still be observed at the present time, five months after they were received. The other colours, both on sheet and crepe, are still as obvious as they were at the beginning; the periods of time for which these samples have been kept varies from four to eight months." Although, therefore, the colours may in some cases tend to fade, it would appear that they are capable of lasting for a considerable period of time.

INVESTIGATIONS ON THE CAUSE OF THE SPOTTING.

Apart from the suggestion which was raised to the effect that the spotting was due to chemical changes in the rubber, all observers quoted above appear to have associated the discolourations with the presence of micro-organisms. It was with the latter point in view that inves-

tigations were commenced for the purpose of examining the origin of the spotting. It was clearly possible to attack the problem from two sources, viz. either to attempt to obtain organisms from the spots, to cultivate them and if possible, to reproduce the spotting by means of them, or else to make an inventory of the organisms found in latex, latex cups, pails, etc. and in the factory and to ascertain whether any of these would produce discolourations in the rubber. The former was selected as being the more direct course to adopt.

PRELIMINARY EXAMINATION OF THE RUBBER.

It was found to be difficult to obtain razor sections of a sufficient transparency to be subjected to microscopic examination. In some very few cases the sections showed contents in the rubber, but in by far the majority no contents were visible.

Another method of examination proved more satisfactory. This consisted in thoroughly cleaning the surface of the rubber with alcohol applied by a brush, cutting a thin section of a spot and dissolving the caoutchouc in xylol on a microscope slide. Benzol may also be used. When the caoutchouc has been dissolved the residue is mounted in Canada Balsam and examined.

Examined by this method red colours on sheet for the most part shewed the residual pigment with small bodies as to the nature of which it was not considered possible to draw any satisfactory conclusions.† In some cases, however, it was found that the red colours on sheet showed on examination what appeared to be branched hyphae of fungi of a reddish colour.

Red spots on crepe usually showed fungus hyphae, but in some cases the hyphae were absent.

The dark-coloured spots frequently showed the presence of what appeared to be brown hyphae; while in some cases the spots contained only masses of dirty brown substance, frequently of mineral or vegetable nature.

Comparative examination of spotted and unspotted parts of the rubber were made and it was found that the structures which resembled fungus hyphae in appearance were associated with the spots and were absent from unspotted parts. In the blue and black spots these structures were in many cases particularly well shown.

† Comparative examinations with *unspotted* sheet also shewed sometimes the presence of small bodies, apparently constituents of the rubber unaffected by the xylol.

For purposes of microscopic examination fresh material showed the contents more clearly than rubber which had been dry for some weeks. In the latter case the structures resembling hyphae were disconnected in small portions, the original filaments having presumably been broken into small bits by the pressure of the cover slip.

In the plates at the end of this paper figures are given illustrating the appearance of the structures in the residue which was left after dissolving the caoutchouc.

Following on the association of the discolourations in some cases with what appeared as microorganisms, attempts were made to obtain growths from the spots and the following methods were employed:—

A fresh spot was cut out of the sheet or crepe, before it had dried, of about ½ sq. cm. in area and was washed in 1 to 1000 mercuric chloride for three minutes and then in sterile water for ten minutes. It was transfered to a glass block which had been recently flamed and was kept covered with a clean bell-jar. The caoutchouc was next cut into thin strips with a sterile knife and the strips were transfered to nutrient media with a pair of sterile forceps. Two media were employed, the one an extract of the juice of the sugar cane† and the other nutrient bouillon + 10. The object in view was to obtain, if possible, a growth of the organism in the medium from the cut surface of the rubber. Several strips of caoutchouc, usually six, were transfered to the nutrient media in each case.

When the caoutchouc, bearing spots which on preliminary examination contained what appeared be to fungus hyphae, was transferred to cane-juice-agar growths of fungus mycelium were sometimes obtained in the medium. In some cases only a single organism was present, emanating from the cut surface of the caoutchouc. In other cases this organism was present along with common moulds which are recognised as saprophytes, *e.g. Eurotium candidum*, *Penicillium glaucum*, etc.; while sometimes no growth of fungus hyphae was obtained. These common moulds were regarded as being contaminators in so far as they developed no colour, and the cultures which showed the growth of a single organism were selected.

† This is employed in the laboratory as a general medium for artificial cultivation of fungi and has been found to be very serviceable. It is prepared by removing the rind of fresh sugar cane and weighing out 200 grm. of the internode. This is cut into thin slices lengthwise and heated with distilled water to 60° or 65° C. for 15 minutes and then to 100° C. for 20 minutes. It is filtered and made up to 1000 cc with distilled water. Agar-agar 1.5% is added. A slightly acid medium is thus obtained which appears to be particularly suitable for the growth of saprophytic fungi.

It was found after numerous trials that in order to obtain a growth of hyphae from the caoutchouc fresh material was required; spotted rubber which had been dry for several weeks did not produce a growth of mycelium. Transfers were made from cultures which contained a single organism.

Material from a diffuse red colour on sheet and also from a red colour on scrap was treated in the way described above, but was tansferred to nutrient bouillon + 10.

Further work on the organisms which were obtained by the above methods from the spotted rubber will be detailed under the heads of the individual organisms.

MONASCUS HETEROSPORUS (Schroter) (?)

When spotted rubber from certain samples of sheet and crepe was tansferred to cane-juice-agar it gave rise to a growth which originated at the cut surface of the rubber and spread through the nutrient medium. This growth which was found to consist of hyphae of a fungus and appeared in most cases within 72 hours after the rubber was transfered to the cane juice-agar.

A red pigment made its appearance usually within five days.

Other samples which showed a similar spotting failed to give rise to any growth.

A preliminary examination showed that the hyphae produced what appeared as reproductive bodies of two distinct kinds. Transfers were accordingly made from these by the usual poured-plate method in cane-juice agar-agar. The growths were reproduced in the transfers and those plates which showed one or two initial growths were selected, the others being discarded.

The hyphae appeared in the transfers after about 48 hours; they were much branched, septate at fairly regular intervals and measured about 3 microns in width. The reproductive bodies appeared in about 4 days. They were of two kinds. Conidia were borne in chains at the ends of lateral branches of the hyphae; the chains were simple and were composed of five or six conidia. In some few cases only a solitary conidium was observed. Perithecia arose simultaneously at the ends of short lateral branches of the hyphae; each consisted of a globose ascus containing an indefinitely large number of spores. The wall of the ascus was invested with hyphae. The stalks of the perithecia

were once or twice septate and frequently produced one or more branches which were curved inwards at the tips.

The conidia were globose and measured 7.5 to 9.5 microns. The perithecia were small, measuring 40-55 microns in diameter. The ascospores were oval and measured 5 x 4 microns.

In water containing .5% cane sugar the germination of the conidia and ascospores was recorded in from 24-36 hours. The conidia emitted one or two hyphae. Each ascospore emitted usually a single hypha.

The pigment was in some few cases absent altogether from the growths; but in most cases a distinct red pigment was produced. The hyphae of the fungus were frequently pigmented; but the reproductive bodies shewed the pigment best. The pigment appeared to be situated in the walls of the conidia, perithecia and ascospores.

In one case a set of three transfers developed a bright yellow pigment. This would indicate that the colour is not constant. These transfers had been kept at the laboratory temperature and under similar conditions to the others from which they were derived. No time was, however, available for making a study of the relation of the red to the yellow pigment.

The fungus was isolated on three occasions, twice from a red spot on crepe and once from a small red spot on sheet. From the characters of the reproductive organs it belongs to the genus *Monascus* and would appear to resemble very closely *Monascus heterosporus* (*Physomyces heterosporus*, Harz) Schroter, a fungus obtained on the refuse of candle manufacture in Bavaria. A small conidial form is, however, described for *Monascus heterosporus* which I have not been able to record.

The nature and reactions of the pigment of *Monascus heterosporus* were studied by Harz who named it *Physomycin*. No time has, however, been available for a detailed study of the pigment to be made in this case. The pigment was found to be more evident in the conidia and perithecia than in the hyphae. It took the form of a diffuse colouring which in older cells was found to be associated with the globules contained in the cells. In the ascospores the pigment was particularly evident.

The pigment is soluble on prolonged soaking of the rubber in alcohol.

It was found that continued cultivation in cane-juice-agar resulted in a loss of pigment production.

The Production of the Colour in Rubber by Inoculating with the Fungus. Two experiments were conducted with a view to ascertaining whether the spots could be reproduced artificially by employing the fungus to inoculate biscuits of coagulated rubber. Biscuits were prepared by coagulating 100 cc. of latex with 10 cc. of 1% solution of acetic acid. The latex had been previously diluted by adding to it one-third of its volume of water. The fungus taken from a pure culture was transferred to the surface of some of the biscuits either by means of a sterilised needle or by spraying the surface with sterile water containing the spores.

Experiment (*1*). Twelve biscuits were prepared. Of these nine were sprayed with water containing spores of the fungus, and three were untreated. They were hung on glass rods in a steam oven for five days, without being heated, and were then removed and hung on a string in the laboratory to dry. Yellowish red spots made their appearance on some of the biscuits as the surfaces dried at the end of about eleven days after their preparation. These superficial spots were small and gradually faded as drying continued. At the end of three weeks four biscuits showed a diffuse red colouring which had extended through their substance, the biscuits being then nearly dry. Of the other five biscuits which had been sprayed with the spores two showed the remains of the yellowish red spots which had almost faded away. The other three showed no discolourations. The control biscuits which had not been sprayed showed no discolourations.

Experiment (*2*). Nine biscuits were prepared. Six were inoculated by stabbing the surface on the day after coagulation with a needle which had been passed through a culture of the fungus. The remaining three were untreated. The biscuits were rolled gently and hung in a steam oven over glass rods as in the previous experiment. They were removed at the end of seven days and were hung on a string in the laboratory to dry. Examined at the end of four weeks when they were nearly dry, it was found that in four of the biscuits a red colouring had developed in the rubber at the point of the stabs, the positions of which were quite evident in the biscuits. The discolourations exhibited a spread from the stab in all directions through the substance of the rubber, the greatest measurement recorded was nearly half an inch from the stab. Two of the inoculated biscuits and the controls shewed no discolourations.

Reisolation of the Fungus from the Discolourations Obtained in Experiments (1) and (2). By the method described previously sections of the spotted rubber were transferred to cane-juice-agar under sterile conditions, the sections being obtained from a discoloured biscuit selected from each experiment just before the biscuits had completely dried. In from three to four days a growth was apparent in most of the cultures, spreading from the cut surfaces of the caoutchouc. In from six to seven days a red pigment appeared in these growths. Eight cultures were made, five from a biscuit in experiment (1) and three from a biscuit in experiment (2). Of these, three produced a growth from experiment (1) and three from experiment (2). The growths when examined miscroscopically were found to contain the conidia and ascospores of a fungus indistinguishable in shape and measurements from those of the fungus which had been employed for inoculating the biscuits. In two cases they were contaminated by moulds, in the remaining four cases they appeared to be pure.

The Growth of the Fungus in the Rubber. The hyphae of the fungus in the rubber are for the most part thinner than those which are obtained in artificial cultures.

In some cases groups of rounded cells have been observed; but in the majority of examinations the hyphae evidently pursue a sinuous course, their walls being wavy in outline.

Both in the discolourations which were produced artificially in the experiments (1) and (2) and in naturally spotted rubber from which the fungus had been isolated the presence of structures which resemble somewhat in appearance the perithecia of the fungus in artificial cultures was demonstrated. These structures in the caoutchouc consisted of a globular mass containing a number of oval bodies resembling spores. Each globular mass was suspended by a hypha. The structures agreed approximately in their measurements with the perithecia which are developed in artificial cultures. The hyphae which form the investment to the ascus could not, however, be distinguished; this may be due to the difficulty in observing the residue sufficiently clearly after the caoutchouc has been dissolved owing the difficulty in obtaining satisfactory staining of the residual mass.

The hyphae in the caoutchouc are shown in plate (1) fig. (1) and the structures which resemble the perithecia in fig (2).

Summary. To summarise the results which are based on the above work, it may be said that :—(1). Examinations of spotted rubber have revealed what appeared as hyphae of a fungus in the residue which was left after the rubber was dissolved. (2). Spotted rubber when transfered to artificial media on three occasions, twice from crepe and once from sheet, gave rise to a growth of a fungus which was in some cultures pure. (3). Material from the pure cultures was used to inoculate biscuits of rubber and in some cases the development of a red colour was produced. (4). From the artificially produced discolouration a fungus which was indistinguishable in appearance from that obtained in (2) above was isolated.

It is, therefore, concluded that the fungus is capable of causing a discolouration of the rubber.

SPONDYLOCLADIUM MACULANS, N. SP.

Isolation from crepe rubber. The fungus was first obtained by employing the method above described from a yellowish-red colour on crepe. More recently, however, it has been isolated from a dark green or almost black spotting on crepe. It has been frequently observed that yellowish-red and dark spotting are closely associated with each other. The reason for this will, perhaps, be evident when an account of the growth of the fungus in artificial cultures is given.

When portions of discoloured rubber were transferred to cane juice-agar they were found to give rise to a growth which was at first of a rose colour. The growth darkened rapidly and from an examination it was found to consist of hyphae of a fungus which when young appeared to be diffused with a pink colour, which was also present in the medium of the culture, and when old to assume a darker colour. The fungus produced three-septate spores on the surface of the medium and these were employed for the preparation of transfers. In the transfers, plates were selected which exhibited a growth either from one or from two points. The growths of hyphae were at first of a rose colour and became rapidly darker. As the growth increased in size there projected from the advancing margin of each a distinct band of a purplish rose colour. An examination shewed that the youngest hyphae on the margin of the growth were infused with a rose colour, which also extended beyond the extremities of the hyphae into the culture medium. It was concluded that since the growth

was pure the hyphae of the fungus were capable of extruding a pigment into the medium in which they were growing.

It would appear possible to explain the isolation of the fungus independently from yellow-red and also from dark coloured spots by similar changes in colour to that of the mycelium which was observed in artificial cultures.

The Characters of the Fungus in Artificial Cultures. The hyphae measured about 3-4 microns in width and were frequently septate. The older hyphae possessed large refractory globules.

Conidia were produced from conidiophores at the surface of the medium, which were dark-brown, nodular at one or two intervals and measured 100-190 x 6 microns. The conidia were produced in whorls at the nodes, up to six in a whorl, were barrel-shaped, three-septate, with distinct constrictions at the two outer septa; the two middle cells of each conidium were dark-brown in colour and the two end cells of a lighter colour. The conidia measured 23-25 x 11-14 microns.

Germination of Conidia. In .5% cane juice in a hanging drop germination was recorded as early as 18 hours. In latex diluted with water in a hanging-drop germination was recorded in about the same time, while in from 5-7 days dark-brown hyphae were visible in the mass.

No inoculations have been carried out with the object of reproducing the colour artificially in rubber by employing the spores of the fungus.

From the characters of the mycelium and conidia the fungus has been designated a new species. Species of the genus *Spondylocladium* have been for the most part recorded on dead vegetable material as saprophytes. One species, *Spondylocladium atrovirens*, is the cause of a disease of the tubers of the potato.

MYCOGONE SP:

During the beginning of 1911 a red flush appeared on two sheets of rubber which were made at the Experimental Gardens at Kuala Lumpur. The discolouration penetrated through the thickness of the sheets.

Thin slices of the discoloured rubber were cut off with a knife and transferred to cut surfaces of sterilised potatoes. In about 5-6 days three of the surfaces showed a pink colour. On examination a fungus was found on the potato consisting of rather large hyphae whose walls were coloured pink. Certain of the hyphae were found to bear spores, a single spore arising at the apex of each hypha.

Transfers were made to a cane-juice-agar medium from the fungus on the potato. The colour of the fungus in the culture was deep red. The hyphae were 3.75-4 microns in width and frequently branched; the pigment appeared to be present in the cell-walls. From the hyphae short branches arose each bearing a single spore at the apex. The spores were two-celled and measured 15-17 x 10-12 microns. Each consisted of a large cell and a small cell, the latter measuring about 5 microns in diameter. The spores are of the *Mycogone*-form and I have accordingly identified the fungus as belonging to the form-genus *Mycogone*. The pigment was particularly evident in the wall of the bigger cell of the spore.

DIPLODIA-FORM (THYRIDARIA TARDA).

A dark blue spotting on crepe rubber on examination by the method described above was found to contain dark-brown hyphae which were large (4 microns wide) and consisted of short cells (pl. III, fig. 1).

By transfering the spotted rubber to cane-juice-agar a growth of black mycelium was obtained which gave rise to pycnidia bearing spores at first colourless and later dark-brown and one septate. The mycelium and spores were indistinguishable in appearance from those of the *Diplodia* form of *Thyridaria tarda*, the fungus which causes a dying-back of the stem of the Para Rubber tree. The fungus occurs commonly on dead plant material of all kinds in this country.

Germination of the Spores. The spores germinated freely in .5% cane sugar and in diluted latex in hanging-drops. In the latex they gave rise to a dark-brown mycelium.

The above fungi have been obtained from spotted rubber and the germination and growth of three in latex has been demonstrated; while one of the fungi has been employed to reproduce the colouring in prepared rubber artificially and has been again reisolated. This would appear to establish that fungi are capable of causing a spotting of prepared rubber.

CLEAR PATCHES ON SHEET.

Clear patches sometimes make their appearance on unsmoked sheet. They contain growths of fungus mycelium producing conidiophores at the surface of the rubber. The fungi are of several kinds, usually species of *Penicillium* and *Aspergillus*.

BACILLUS PRODIGIOSUS.

Certain red discolourations, occurring on sheet, on bark crepe and on scrap, on examination showed after the solution of the caoutchouc a red pigment with which were associated small bodies resembling bacteria in appearance. These bodies were, however, not always easily distinguishable. Fungus hyphae were absent from these discolourations.

Strips of discoloured scrap were cut, after the outer surface had been sterilized by the method described above and were transfered to tubes of nutrient bouillon : 10. The bouillon became cloudy in about 12 hours and a pink ring was visible at the surface of the liquid on the glass in about 24 hours. From this bouillon plates were poured in nutrient bouillon agar-agar by the dilution method. Colonies of a bacterium were obtained and were propagated by transfers. Their growth was studied also on potato cylinders and in neutral bouillon. The results of the observations are as follows:—

Structure. A small coccoid or short rod-like bacillus measuring up 1 micron, examined in neutral bouillon after two days.

Motility. Examined in hanging-drop the organism was found to be motile.

Nutrient Bouillon Agar-Agar. Red colonies appeared within 24 hours. In the medium the growth of the colonies appeared to be slow; on the surface it was more rapid and the colonies were of a brighter red.

On an agar slant the red colour developed in about 24 hours, becoming rapidly deeper as the cultures became older.

Potato. On potato cylinders a white growth appeared in from about 14-18 hours. The growth changed rapidly to a red colour. A luxuriant growth was produced.

Milk. A red colour appeared at the surface. A coagulum was produced within 60 hours.

Nitrate bouillon. Tested after three days with metaphenylene diamine a deep colour was obtained, the controls showing no reaction.

Pigment. Cultivated at 37½ °C some of the cultures shewed a trace of pigment, the majority being colourless. On prolonged soaking of the caoutchouc in alcohol the pigment was dissolved.

Gram's Method. The organism did not stain by Gram.

Odour. The potato cultures developed a distinct odour of methylamine and ammonia.

Dr. Fletcher, Pathologist to the Medical Institute, Kuala Lumpur, examined the reactions of my cultures and compared them with the ordinary reactions of *Bacillus prodigosus*. He obtained a liquefaction of gelatine by the pink growth and similar reactions to those given by a typical *B. prodigisus*, except that there was a trace of pigment at 37 °C.

The cultures which were isolated from sheet, bark crepe and from scrap exhibited similar reactions and were concluded to be identical with each other. The organism is accordingly regarded as being *Bacillus prodigiosus*.

Experiments on inoculation of the rubber. (1) Six biscuits were prepared by coagulating in each case 100 cc. of latex with 10 cc. of a 1% solution of acetic acid. When the clot had formed four were inoculated by means of a sterile needle which had been passed through a culture of the bacillus; two were untreated. The biscuits were rolled and hung up to dry. At the end of the third day the surfaces of all of the four biscuits which were treated showed a distinct red colour. The colour became more obvious as the rubber dried. The controls showed no colour.

(2). Four hundred cc. of latex were inoculated with the bacillus and coagulated in lots of 100 cc. each, being rolled into biscuits as above. Two hundred cc. of latex were not treated and were employed to make two biscuits for controls. The results obtained were as in (1), shewing a rapid diffuse colouring of the rubber.

Re-isolation of the bacillus. One coloured biscuit from experiment (1) was selected and treated as on p. 11, the cut portions of caoutchouc being transfered to neutral bouillon in tubes. The bouillon was clouded in about 12 hours. This was followed by the appearance of a pink ring at the surface of the liquid on the tube and an accumulation of sediment at the bottom of the tube. On examination a small motile bacillus was detected. This was plated out in nutrient bouillon agar and cultivated on potato cylinders. It gave similar reactions to the tests as applied above.

Accordingly, the organism was regarded as being capable of producing a discolouration of prepared rubber.

OTHER ORGANISMS.

More recently attempts have been made to isolate organisms from a yellow spotting on crepe and have resulted in a species of *Sterigmatocystis* being obtained, which possesses a yellow coloured mycelium giving rise to a bright yellow growth in culture media.

A red yeast (unidentified) has been obtained on one occasion from a red spotting on sheet rubber.

No attempts have been made to reproduce the spotting with either of the above organisms.

In addition, it is possible that other bacteria, *e.g.* Sarcinae may be capable of including spotting in prepared rubber.

THE OCCURRENCE OF THE ORGANISMS IN NATURE AND THE POSSIBLE SOURCES OF INFECTION OF THE RUBBER.

Of the fungi which have been described above the Diplodia-form is best known. It is widely distributed in this country on recently dead vegetable matter, including dead shoots of plants, recently dead wood and fruits and vegetables of all kinds. It is easily transmitted by spores which may be carried by wind. The fungus occurs on estates on prunings and other dead branches and trunks and roots of Hevea and produces its spores readily, only 7 days being necessary for a crop of spores to be developed from the growth of a single spore. The spores exist in the air in quantity as is shown by the ease with which growths of the fungus may be obtained by exposing cut surfaces of potato or pineapple to the air. It is accordingly not difficult to conceive the method by which they may be introduced into latex either before or after coagulation.

Poles of uncured jungle wood have been sometimes employed in drying houses for hanging rubber and these have been found to bear superficial growths of fungus mycelia. By paring off thin strips of the poles and introducing them into nutrient media, growths of three of the above fungi have been obtained, *viz*: *Monascus heterosporus*, *Spondylocladium maculans* and the *Diplodia*-form. *Spondylocladium maculans* appears to be particularly common on the poles, giving rise to superficial black or reddish-yellow discolourations. The presence of the *Diplodia*-form can be identified by the greyish discolouration of the substance of the wood which is evident when the interior of the wood of a branch affected by this fungus is exposed.

The above observations offer a possible explanation of a phenomenon whose nature was for some time quite obscure, *viz*: the distribution of the spotting on crepe rubber in transverse bands recurring at intervals. It was not possible to suggest any explanation of this regular distribution

of colour until the fungi had been isolated from the racks on which the crepe was hung. On two occasions *Spondylocladium maculans* and the *Diplodia*-form were obtained both from the wood of the racks and from the crepe which was in contact with the wood. Each portion of the crepe which was in contact with the wood was discoloured bluish or black and reddish-yellow. The occurrence of the spotting at intervals is explained by the alteration in the position of the crepe as drying progressed, thus presumably exposing a fresh surface to infection by contact with the wood of the rack.

The two fungi *Spondylocladium maculans* and *Monascus heterosporus* are propagated by conidia, and the latter by spores which are produced in an ascus. The conidia are of a type capable of transmission through the air.

From the above the infection may be considered to occur either in the field or, after the rubber has been rolled, in the drying-house. In the case of the fungus *Monascus heterosporus* it was demonstrated that infection could occur either during coagulation or after the biscuits were prepared. The influence of 0.1% acetic acid which was employed for coagulating the biscuits in the experiment on p. 14 evidently had no effect on the growth of the fungus. After the rubber has been rolled the greater part of the acid is removed.

The possibility of infection occurring either in the field by transmission of the spores of the fungus to the latex or in the drying-house may therefore be established.

Of the occurrence of the species of *Mycogone* which was isolated from sheet rubber little or nothing is known. It belongs to a genus of which some species occur as parasites on other fungi.

The species of *Sterigmatocystis* referred to above is a common mould, living on dead vegetable matter, etc. It is one of the fungi which cause what is popularly known as "mildew" on articles of food, clothing etc. The spores of the fungus occur in the air in abundance.

In the light of the above reasoning the occurrence of spots only on rubber from one or two fields on an estate would possibly indicate that infection in such cases was derived from the field rather than in the factory.

The bacillus, *B. prodigiosus*, which has been isolated from a red colour on sheet, bark crepe and scrap has been known for a very large number of years to produce a red colour on bread, meat, and other articles of diet. There is a record as early as 332 B. C. when the so-called "blood-

miracle'' which is attributed to this bacillus occurred during the conquest of Tyre by Alexander the Great; the bread of the besieging army was found to red on being broken, a phenomenon whcih was interpreted to mean that a bloody fate awaited those inside the city. The bacillus was responsible for the ''Wunderblut'' of the Middle ages. In the nineteenth century there were three notable epidemics of this red colouring of food in Europe; one occurred in Padua, Italy, in 1819, one in 1844 among the food supplied to the French army and the third in Berlin in 1848 when Ehrenberg described the organism and named it prodigiosus. The organism is very widely distributed in nature and is one of the best known of the colour-producing saprophytic bacteria. It is commonly found in ordinary drinking water.

The occurrence of the red colouring on scrap before it is removed from the tree has been noticeable on several occasions on different estates, the bacillus having been isolated from the coloured scrap. This indicates outside infection due in some cases possibly to water which is placed in the tapping cuts to induce a flow of latex. The water may also provide a source of infection for the latex. Infection may also occur through the air.

The bacillus propagates itself rapidly and has been observed to spread with readiness from sheet to sheet in a drying-house. There are records in which the discolourations have appeared on rubber within 3 or 4 days after it was rolled.

Spotting due to the bacillus usually takes the form of an epidemic which may affect only one or two weeks' output of rubber and then disappear for some time.

OTHER CAUSES OF DISCOLOURATIONS.

Oil is not uncommonly a source of discolouration in rubber. The dark-brown or black streaking on crepe, more especially at the sides, is commonly due to the oil from the rollers during machining.

Oil and dirt can be recognised in microscopic examination by the presence of globules and portions of mineral and vegetable matter in the residue which is left after the solution of the caoutchouc.

Blackening or darkening of the rubber which is attributed to chemical alteration of bodies in the latex is well-known and need not be detailed here.

METHODS OF TREATMENT.

Under this heading different methods which may be useful in preventing the occurrence of spotting will be treated with. It is essential that the cause of the discolouration should be ascertained before any preventive treatment can be applied. For example, from the above it appears that discolouration may be due to a fungus growth or may be bacterial, or it may be due to oil or to oxidation; and different measures should be adopted in each case.

Drying. It has been observed that where drying-houses occur in damp low-lying situations or are surrounded by trees or other buildings the rubber dries slowly and spotting from fungi is frequently common. Similarly where the facilities for drying in the building are not adequate, *i.e.* where good access and good circulation of air is not obtained, spotting may be frequent.

Reviewing some of the forms of prepared rubber which develop spotting, thin crepe appears to be less susceptible to spotting than thick crepe; while artificially-dried crepe, as far as has been observed has not been found to become spotted. Other things being equal the thicker the crepe the longer will it take to dry. By artificial means a very rapid drying is effected. Again, steamed crepe which takes a comparatively long time to dry appears to be very susceptible to spotting.

No organism can grow if there is not sufficient water present. The minute fungi contain a large percentage of water; it is little wonder, therefore, that they cannot vegetate under dry conditions. One of the conditions which favours the development of such fungi is a damp, still atmosphere. It would appear, therefore, that rapid drying should diminish the quantity of spotted rubber. The spotting caused by fungi takes some time to make its appearance owing to the time necessary for a growth of the organism to be accumulated sufficient to produce a discolouration. Accordingly one would expect that rapid artificial drying which would be completed in a few days should altogether prevent the development of this spotting. This is known to be the case.

It is probable that in artificial drying the high temperature obtained may be fatal to the spores of the fungi which induce spotting. This may obtain in cases in which infection was derived in the latex previous to coagulation. In addition to the absence of spotting great economy of time is effected by rapid artificial drying of the rubber. It is

possible, however, that the fluctuation in the form of rubber which meets with favour on the market has prevented the adoption of artificial drying on a larger scale.

Where fungus spotting is frequent on crepe it would be advisable to increase the rate of drying by any required improvements in the building or its surroundings and by avoiding wherever it is possible overcrowding in the building, *i.e.* ensuring a good drying space for the rubber. The crepe may also be rolled thinner, where it is practicable, so as to ensure its drying more quickly.

Smoking. Spotting due to fungi and bacteria has not been found on smoked rubber. In some cases rubber which has developed spotting has been smoked after the spots appeard. This does not appear to obliterate the spots in most cases, but it renders them less evident.

Smoking appears to have two separate actions in preventing spotting. In the first place some of the constituents of the smoke act as antiseptics. In the second place smoking ensures a rapid surface drying. How far smoked rubber owes its preservative qualities either to one or the other is not known; but it is not doubted that both agents are here at work.

Although ordinary smoke possesses antiseptic properties it is not generally regarded, as used in the preservation of food, as being powerful enough to prevent bacterial and other growths. When, however, fuel rich in creosote and phenol compounds is employed the case is different.

The Application of Chemicals. Following on the results obtained above and the conclusions based on them with respect to the causes of spotting of prepared rubber, the application of poisons to the rubber either before or after coagulation suggested itself naturally as a means of prevention worthy of trial. The poisons which are commonly employed to prevent the growth of parasitic fungi are compounds of copper, mercury, sulphur, along with potassium permanganate. Of these, copper salts produce a peculiar toxic action on rubber and are said to be effective even in traces; mercury salts are said to cause a darkening by the formation of organic mercury compounds in the rubber, while a further blackening may occur by the production of mercury sulphide on vulcanisation; sulphur compounds would presumably be open to objection by reason of the employment of sulphur in vulcanisation; the use of potassium permanganate is also inadvisable. This disposes of the use of the above chemical compounds as possible preventives of spotting.

In addition to the above there is the objection which may possibly be raised by the manufacturer to the use of any metallic salt; and, even if the salt were employed in very small quantities so as to give no indication of its presence when chemically tested, the fact that metallic salts were used in plantation rubber might tend to bring the rubber into disfavour on the market.

For similar reasons salts of iron, arsenic, lead, etc. may be discarded.

Carbolic acid cannot be employed owing to its dark colour.

This leaves reagents of the type of formaldehyde. Formalin, which is a 40% solution of formaldehyde, has been been employed in latex for preventing coagulation. Its action in this respect it owes, perhaps, to its killing of the bacteria in the latex. One part of formalin in four hundred of latex would probably be effective in sterilising latex when brought from the field to the factory. The cost of formalin in London is about 55 - a hundredweight. One pint of formalin in 50 gallons of latex should be effective.

In the preparation of the crude rubber the washing and rolling to which the coagulated latex is subjected would be expected to get rid of the greater part of a soluble chemical compound. It may, therefore, be expected that while the use of formalin for sterilising latex may be advantageous in preventing the development of spotting from outside field infection its further action may be so reduced after the washing and rolling of the rubber as to render it negligible.

FUMIGATION OF DRYING HOUSES.

There are methods in common use for disinfecting the insides of buildings either by fumigation or by washing the floor and wood work with an antiseptic. Formalin is employed as a spray or its vapour is produced by the aid of special contrivances, such as the "para-form lamp," in which para-form is subjected to the action of the vapour produced by the combustion of methylated spirit. The objection to the use of formalin as a spray is its irritating effect on the eyes and on the mucous membrane of the throat and nose, and consequently it must be applied with care; 1 part in 400 water should be effective.

The fumes of burning sulphur are employed to disinfect buildings, the floor and wood-work of which has been previously washed with water. The quantities recommended

are about 6 pounds of sulphur to 1000 cubic feet of space, the building or room being kept closed for 24 hours. Cylinders of liquid sulphur dioxide are also obtainable in various sizes and are employed for fumigating buildings.

Fumigation of large drying-houses is in most cases scarcely practicable owing to the necessity for removing the rubber previously and the absence of outside accomodation. Where outside accomodation can be provided for the rubber it might be advantageous to disinfect the building periodically when the spotting is abundant.

The use of formalin requires great care. The use of Condy's fluid is undesirable owing to the action of the manganese. The means of sterilisation which suggests itself as being most suitable is by means of sulphur fumes. It would be necessary to repeat the disinfection of the building at intervals. A further difficulty in the employment of sulphur dioxide is the question of closing the building so as to render it almost airtight.

Methods of sterilisation of the insides of drying houses would not be expected to have any lasting effect owing to the building being again opened to the introduction of the spores of the organisms from outside. It is doubtful as to how far there is an appreciable spread of the spots from one piece of rubber to another in the drying-house, except in the case of the red spotting caused by *Bacillus prodigiosus*, providing the sheets of rubber are not in actual contact with each other. Ordinary examinations do not reveal the presence of spores of the fungi on the surface of the rubber. The ascospores of Monascus are believed to be produced in the substance of the rubber, but this does not lend them to active aerial transmission. It would appear likely that much of the spotting at any rate is derived from outside infection, *i.e.* from spores introduced into the latex from the field or transferred to the factory by the air.

ISOLATION OF SPOTTED RUBBER.

The same considerations apply here as do in the case of the disinfection of buildings. The removal from the building and isolation of spotted rubber is frequently impossible owing to the absence of outside accomodation for any large quantity of rubber.

Where spotting in a drying-house is caused by *Bacillus prodigiosus*, all spotted rubber should be isolated as early as possible, whenever it is possible to do so, as the rapid spread of the infection from one sheet to another is well-

known in this bacillus. Again, sheet or crepe which is spotted by the bacillus should not be packed among other rubber for shipment.

When spotting is due to fungi, isolation may not be so necessary as in the case of the bacillus, but it should be carried out whenever possible as soon as the spots appear.

The development of spotting while the rubber is in transit was for some time doubted since in the drying-house there is no development of spots after the rubber has dried. More recently it has been fairly satisfactory established that rubber which was packed under most careful supervision was found to be spotted on arriving in London. Actual experiments on this have not been carried out, but an accumulation of evidence would indicate that it can sometimes occur. Presumably the conditions of dampness necessary for the germination and growth of the spores of the fungi prevail while the rubber is on the voyage.

OTHER CONSIDERATIONS.

Racks which are constituted of poles of green wood should not be employed in a drying-house owing to the liability to infection of the rubber from the fungi which grow on the poles.

Tables employed in the factory for wet rubber should be kept clean.

Recently dead material in the field should be burnt, *e.g.* prunings, etc., as this harbours saprophytic fungi which may be introduced into the latex or factory.

In the case of *Bacillus prodigiosus* the infection in the field is often obvious owing to the red colour of the scrap on the tree. In such cases it would be advisable to isolate the latex from fields which show red scrap and to treat it with formalin before coagulation. The coloured scrap should be destroyed or kept away from the drying house.

It is always advisable to locate any field or fields from which infection may be introduced to a drying-house and to attempt to ascertain whether there is any cause of infection in the field. It was pointed out in a previous section that water which was employed for commencing the flow of latex or for use in the latex cups might introduce the bacillus. This is a practice which it may be difficult to discontinue and as an alternative to locate infected fields and to treat with formalin the latex derived from these would, perhaps, be preferable.

The remedial measures for oil and dirt are obvious and need not be given.

THE TREATMENT OF RUBBER AFTER THE SPOTS APPEAR.

The actual influence of the discolourations on the market value of the rubber appears to have varied. Some records would indicate that spotting has considerably reduced the market value; while in some cases little attention appears to have been paid on the market to spotting. It is, however, difficult to collect reliable information on the subject.

It apears to be recognised by experts that the growth of microorganisms does not cause any depreciation in the quality of the rubber. Homogeneity in plantation rubber is, however, what is required by the manufacturer, and homogeneity of colour as well as of other physical properties is desirable.

The market value of the better grades of plantation rubber may be expected to be more liable to depreciate from spotting than that of the lower grades.

It would be advisable, when possible, to avoid packing spotted with clean rubber for shipment.

A method of dealing with spotted rubber which appears to give satisfactory results is to allow it to dry and then to crepe it and wash it thoroughly, finally rolling it thin. The reason for this is that the discolourations can usually be washed out after the rubber has dried. In the case of very dark or black spotting this treatment does not always yield satisfactory results; but in lighter spotting it has proved to be quite satisfactory in many cases.

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Agricultural Bulletin, F. M. S., Vol. I, No. 2, 1912, p. 30.

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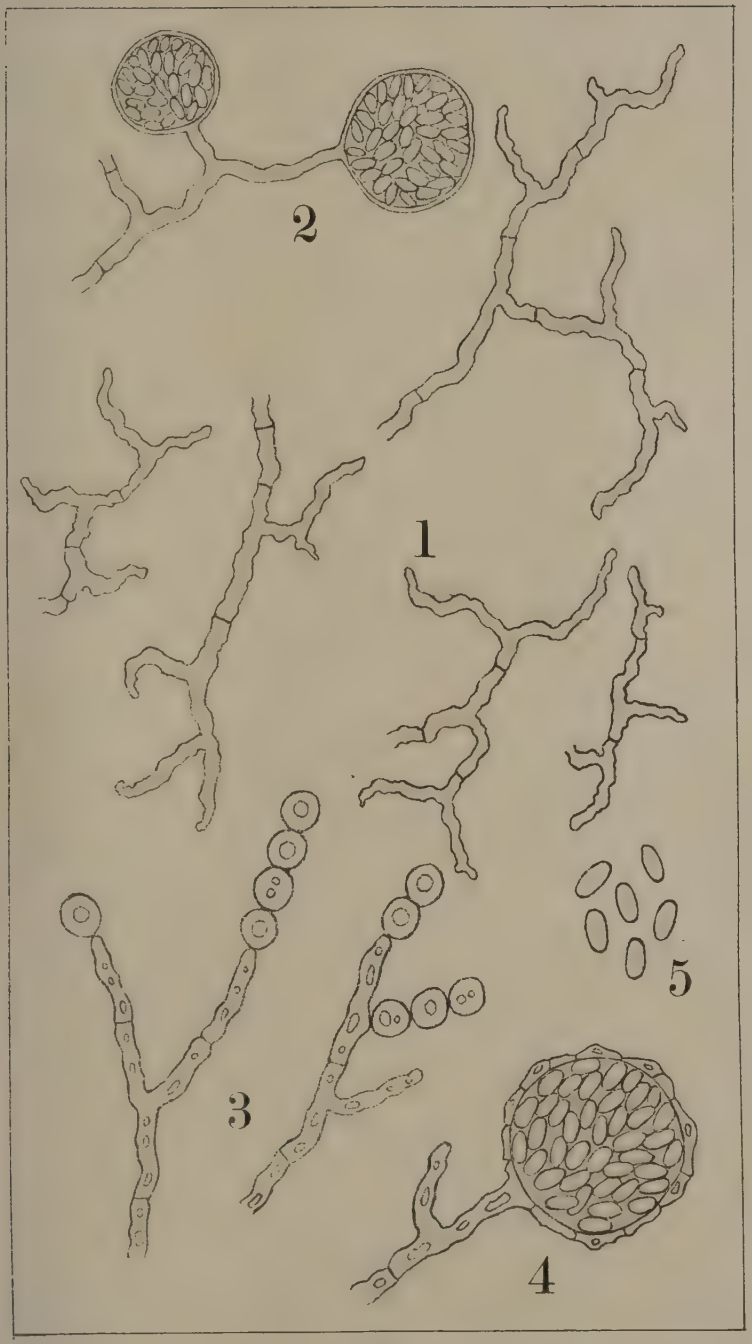

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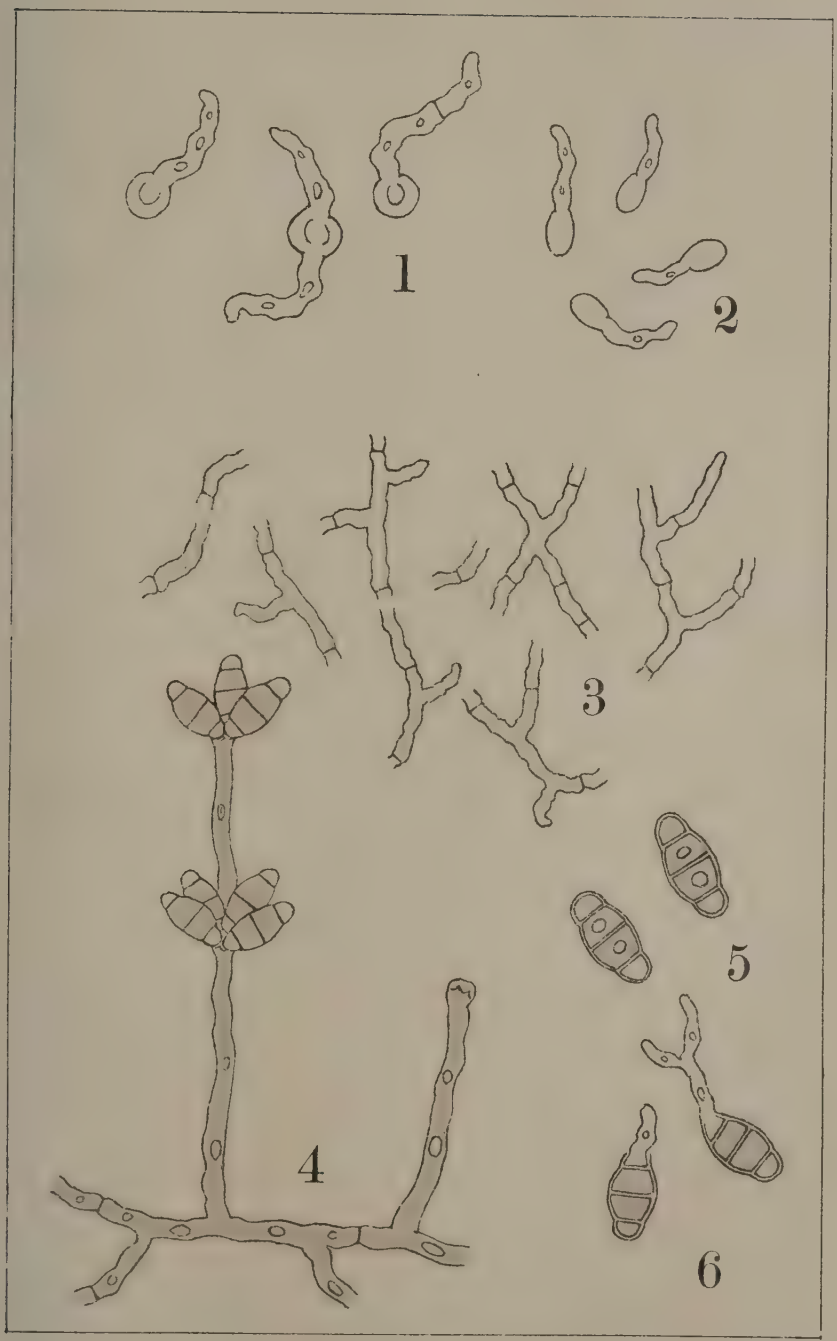
Plate I.



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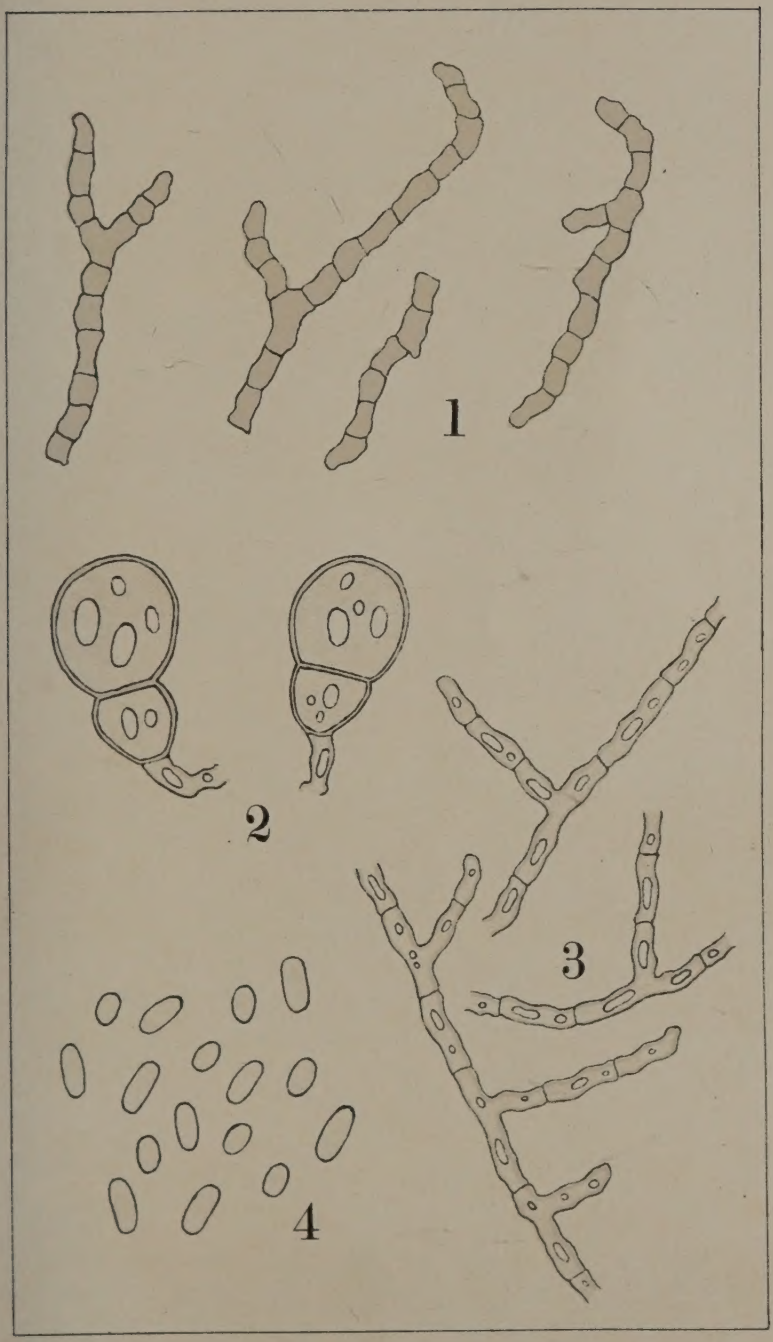

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Plate III.

The following Bulletins have been published by the Department of Agriculture.

1. Notes on *Termes Gestroi* and other species of *Termites* found on Rubber Estates in the Federated Malay States by H. C. Pratt, Government Entomologist.

2. Root Diseases of *Hevea braziliensis*, the Para Rubber Tree: by W. J. Gallagher, Government Mycologist.

3. Observations on *Termes Gestroi* as affecting the Para Rubber Tree, and methods to be employed against its Ravages: by H. C. Pratt, Government Entomologist.

4. A Lepidopterous Pest of Coconuts, *Brachartona catoxantha Hamps*, (Zygaenidae): by H. C. Pratt, Government Entomologist.

5. The Extermination of Rats in Rice-Fields: by W. J. Gallagher, Government Mycologist. *out of print*

6. A Preliminary note on a Branch and Stem Disease of *Hevea Braziliensis*: by W. J. Gallagher, Government Mycologist. *out of print*

7. Coffee Robusta: by W. J. Gallagher.

8. The Cultivation and Care of the Para Rubber Tree (in Malay).

9. Die-Back Fungus of Para Rubber and of Cacao: by Keith Bancroft, Assistant Mycologist.

10. A Lecture on the Para Rubber Tree: by W. J. Gallagher. *out of print*

11. Coconut Cultivation: by L. C. Brown, Inspector of Coconut Plantations.

12. Padi Cultivation in Krian: by H. C. Pratt, Government Entomologist.

13. A Root Disease of Para Rubber, *Fomes Semitostus*; by Keith Bancroft, Mycologist.

14. The Die-back Disease of Para Rubber and a Note on the Leaf Diseases of Para Rubber: by Keith Bancroft, Assistant Mycologist.

15. Camphor: its Cultivation and Preparation in the Federated Malay States, by B. J. Eaton, Agricultural Chemist.

16. The Spotting of Plantation para Rubber, (A Preliminary Account of Investigations on the cause of the Spotting): by Keith Bancroft, Mycologist.

17. The Preparation of Plantation Para Rubber, by B. J. Eaton, Agricultural Chemist.